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Rapid Diagnosis of *Histoplasma capsulatum* Endocarditis Using the AccuProbe on an Excised Valve

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***Histoplasma capsulatum* is an infrequent but serious cause of endocarditis. The definitive diagnosis requires culture, which may require a long incubation. We demonstrated the ability of the *Histoplasma capsulatum* AccuProbe to accurately identify this organism when applied directly on an excised valve that contained abundant yeast forms consistent with *H. capsulatum*.**

Histoplasma capsulatum is a common cause of infection in the midwestern and southeastern United States. This fungus causes a broad array of clinical manifestations, and the diagnosis depends on a high index of suspicion. Furthermore, the presence of small budding yeast in tissue is suggestive but by no means diagnostic of this organism. The diagnosis of histoplasmosis is confirmed by isolation of *H. capsulatum* from body fluids or tissues. Unfortunately, *H. capsulatum* is a slow-growing dimorphic fungus that requires special media for recovery and may take up to 4 weeks to grow (range, 1 to 4 weeks) (8). If *H. capsulatum* is suspected in culture because of its characteristic tuberculate macroconidia, confirmatory testing is required to exclude the possibility of a contaminating environmental mold with similar conidia such as *Sepedonium* spp. Although mycelial-to-yeast-form conversion and exoantigen testing may be used to confirm a mold as *H. capsulatum*, today the *Histoplasma capsulatum* AccuProbe (GEN-PROBE, Inc., San Diego, Calif.), a DNA probe that recognizes a specific rRNA target sequence, is commonly used for more rapid and accurate results (5).

The occurrence of endocarditis secondary to *H. capsulatum* is rare. There are only a few cases reported in the literature (1, 2, 4). The characteristic histopathologic features of *H. capsulatum* endocarditis are clusters of yeast embedded in a fibrin mesh (3). In addition to the small yeast (2 to 5 μm in diameter) typical of *H. capsulatum*, rudimentary hyphal forms and enlarged yeast forms may be seen (7, 11). Although the histopathologic diagnosis of *H. capsulatum* endocarditis is strongly suggested by this morphology, confirmation by culture is necessary. However, the application of molecular methods directly to the excised tissue could potentially achieve an accurate diagnosis without the delays needed for culture.

A 58-year-old man from the midwestern United States, with a medical history of previous hypertension, was transferred to our institution for acute ascending aortic dissection. He underwent an emergent open-heart surgery with replacement of the ascending aorta and the aortic valve with a Hemashield and Carpentier-Edwards valve, respectively. His postoperative course was complicated by acute renal failure, rapid atrial fibrillation necessitating cardioversion, cardiac tamponade requiring a redosternotomy, and *Pseudomonas aeruginosa* mediastinitis with subsequent bacteremia necessitating mediastinal reexploration, abscess drainage, and prolonged intubation with subsequent tracheostomy. After a 2-month convalescence, he recovered well and was discharged to a rehabilitation facility on oral ciprofloxacin for lifelong suppression of the *Pseudomonas* infection. After a few months, he noted weight loss, fatigue, and anorexia. Six months later, he was admitted to a local hospital with worsening of these symptoms, in addition to high-grade fever and chills. Blood cultures grew *P. aeruginosa* that was resistant to ciprofloxacin. A transthoracic echocardiogram was performed that showed large vegetations on the prosthetic aortic valve and mild regurgitation. He was transferred to our institution, where he underwent an aortic valve replacement with a homograft for presumed *Pseudomonas* endocarditis. The histopathology of the excised valve, however, demonstrated large vegetations that consisted of a mixture of yeast forms and fibrin (Fig. 1). Although most of the yeast forms were small (2 to 5 μm in diameter), occasional large,

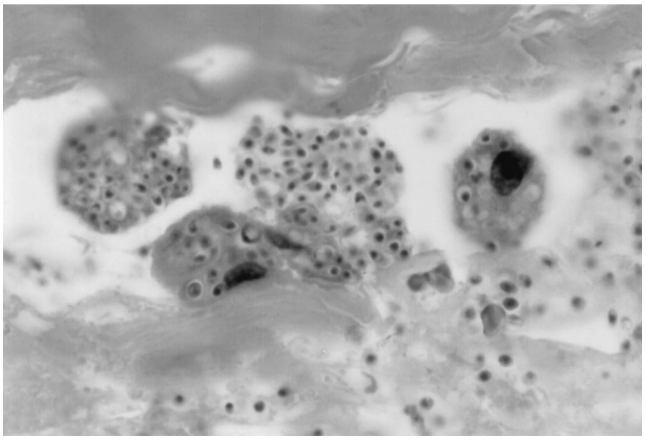

FIG. 1. Small yeast (2 to 5 μm in diameter), as typically seen in histoplasmosis, are present in phagocytes in this vegetation. Hematoxylin and eosin strain. Magnification, ×1,000.

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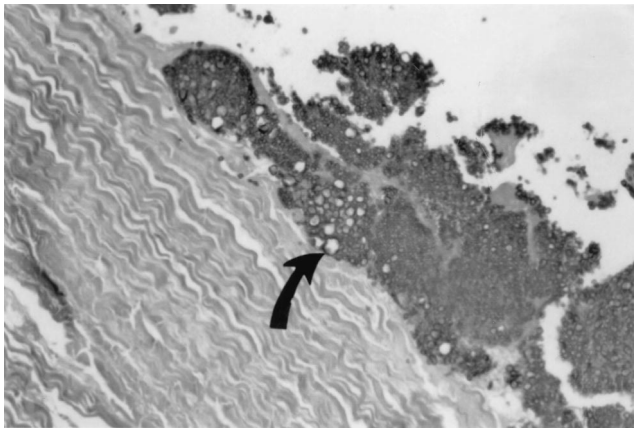

FIG. 2. Enlarged, globose yeast forms (arrow), adherent to the cardiac valve, are best seen using a silver precipitation stain. Grocott-Gomori methenamine silver stain. Magnification, ×200.

globose forms were also seen. The morphology of the yeast was best demonstrated using the Grocott-Gomori methenamine silver stain (Fig. 2). These findings were thought to be most consistent with endocarditis caused by *H. capsulatum*. The portion of valve received for culture was macerated. Direct examination using Calcofluor white-KOH disclosed numerous small budding yeasts. Cultures for bacteria and fungi were performed. Two days later, the valvular tissue grew rare *P. aeruginosa* and a rare anaerobic nonsporeforming gram-positive bacillus, which was considered as a possible contaminant.

We attempted to identify the fungus present directly from the excised valve, using the *Histoplasma capsulatum* AccuProbe, because of the abundance of the yeast seen and the suggestion that this probably represented *H. capsulatum*. Although the efficacy of this test has not yet been demonstrated on direct clinical specimens, the amount of fungus necessary to generate a positive result from culture is minimal (1 to 2 mm^2), and this amount was judged to be present based on the histopathologic examination of the specimen. This test requires less than 1 h to perform, and the results are based on hybridization between a specific chemiluminescent DNA probe and rRNA sequence unique to *H. capsulatum*. The efficiency of hybridization is determined by light generation and was measured in a luminometer (Leader 450 *i*; GEN-PROBE, Inc.). Signals of ≥50,000 relative light units (RLU) are positive.

A 5-mm^2 piece of the surgical specimen was sonicated for 20 min and then heated for 10 min at 95°C. With the exception of a longer sonication (5 min more than the usual time), the *Histoplasma capsulatum* AccuProbe procedure was performed as specified by the manufacturer. The signal produced for *H. capsulatum* in our specimen was 153,961 RLU, which was strongly positive. As a negative control, the same amount of tissue from the valve was tested using the *Coccidiodes immitis* AccuProbe and generated only 17,539 RLU, clearly negative. Of concern, red blood cells that can be present in a clinical specimen have been reported to yield false-positive probe results because of nonspecific chemiluminescence (10). This potential problem was ruled out in our experiment with the use of the *Coccidiodes immitis* AccuProbe as a negative control. One week later, the culture grew *H. capsulatum*, which was also confirmed with the *Histoplasma capsulatum* AccuProbe.

The valvular vegetations in patients with *H. capsulatum* endocarditis, like those produced by other fungi, are large, verrucous, and friable and contain abundant organisms. Our findings suggest that the *Histoplasma capsulatum* AccuProbe may be used for the rapid identification *H. capsulatum* in excised tissue if a sufficient number of organisms and histopathology suggestive of this organism are present. Since the sensitivity of the AccuProbe on a direct specimen has not been shown to be equivalent to that of culture methods (6, 9), additional studies are necessary to clarify the limits of detection (potential sample requirements, clinical relevance, etc).

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